

Effect of Major Spices in Lebanon Bologna on Acid Production by Starter Culture Organisms

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ABSTRACT

The effect of a Lebanon bologna spice mixture and its major component spices, black pepper, allspice, and nutmeg, on acid production by a mixed starter culture containing *Lactobacillus plantarum* and *Pediococcus cerevisiae* was studied in a liquid medium. These spices stimulated acid production by the starter culture organisms although some Lebanon bologna component spices are known to have antimicrobial properties. The spice mixture stimulated *L. plantarum* more than *P. cerevisiae* when each organism was cultured singly. Stimulation of acid production could not be attributed solely to differences in bacterial numbers as defined by plate counts.

The microbiology of spices used in sausages and other meat products has been studied primarily from a viewpoint of their contribution to the contaminating microflora of a product or their inhibiting effects on organisms of public health significance which might be present in a food product. Jensen et al. (5) implicated contaminants from coriander and white pepper in spoilage of canned chopped hams. Castell (2) reported that 20 samples of spices which he examined were heavily contaminated with aerobic thermophiles. In a more comprehensive study of the role of spices in pickled-food spoilage, Fabian et al. (3) found bacterial plate counts in whole and ground spices ranging from 0 to 6.7×10^7 per g, with only cloves and ground cinnamon inhibiting bacterial growth. As food processing techniques advanced and the importance of other organisms of public health significance (*Vibrio parahaemolyticus*, *Bacillus cereus*) was recognized, spices came under renewed scrutiny. Powers et al. (8), reporting on the microbiology of spices procured by the military, found *Clostridium perfringens* in 15% of 115 samples of the seven different spices analyzed. In a later study, Powers et al. (9) found *B. cereus* in 53% of 110 samples of seven different spices. Farbood et al. (4), in a study of the bacteriostatic and bactericidal effects of rosemary spice extractive (RSE) on microbes associated with mechanically deboned poultry

meat, turkey breast, and beef, reported that 0.1% RSE exerted a definite bactericidal effect on *Staphylococcus aureus*. Julseth and Deibel (6) reported inhibition of growth of *Salmonellae* by allspice, cassia, onion, and oregano, and Beauchat (1) found dried oregano and thyme to be highly toxic to *V. parahaemolyticus*.

Lebanon bologna probably evolved from sausage formulations brought to the Lebanon, Pa., area by the earliest Moravian and Palatine German settlers. Factors involved in production of this highly spiced and smoked fermented sausage are currently under investigation in our laboratory (7,11). We observed (10) that acid production by lactic acid bacteria during fermentation of Lebanon bologna decreased when spices were omitted from the sausage formulation. This indicated that spices might play a role beyond that of exerting germicidal effects or contributing a different microflora in the processing of Lebanon bolognas and similar fermented sausages.

This paper describes our research on the effects of Lebanon bologna spice mixture and its major component spices — black pepper, allspice, and nutmeg — on acid production in a liquid medium by a starter culture containing both *Lactobacillus plantarum* and *Pediococcus cerevisiae*.

EXPERIMENTAL

Spices

Purified spices (Griffith Laboratories, Inc., Union, N.J.) were used throughout the experiment. A Lebanon bologna spice mixture was prepared according to the formulation of Palumbo et al. (7): black pepper, 25.0 g; nutmeg, 12.5 g; allspice, 12.5 g; red pepper, 6.2 g; cloves, 6.2 g; cinnamon, 6.2 g; ginger 6.2 g; mustard, 6.2 g; and mace 0.2 g. Total aerobic plate counts of the purified spices determined by conventional plate count methods were less than 100 cells/g.

Liquid medium

Beef extract (Difco Labs, Detroit, Mich.) 3 g; tryptone (Difco), 5 g; sucrose, 20 g; and glucose, 20 g; were dissolved in 1 liter of distilled water. The pH of the solution was adjusted to 6.4 with 0.1 N H_2SO_4 to give a post-sterilization pH of 5.8-6.1. Aliquots of 250 ml of the medium

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were dispersed into 500-ml Erlenmeyer flasks and sterilized for 15 min at 15 psi.

Starter culture

The starter culture used in our fermentation work was Lactacel MC (Merck and Co., Inc., Rahway, N.J.) containing *L. plantarum* and *P. cerevisiae*. In some experiments the individual organisms were used: *P. cerevisiae* (Lactacel, Merck and Co.) and *L. plantarum* (Lactacel DS, Merck and Co.).

Fermentation

Purified spices were added aseptically to flasks of sterile medium to provide concentrations of 4, 8, and 12 g/l, respectively, and 2.5 ml of commercial starter culture diluted with 0.5% peptone water was then added to each flask and to a control containing no spice to give an initial bacterial population in the range of $1.0\text{--}5.0 \times 10^4$ cells/ml. The flasks were incubated for 4 days at 35 C. Samples for bacterial counts and titratable acidity were taken at 24-h intervals.

Bacterial counts

Bacterial counts were made by conventional pour plate techniques with tryptone glucose extract agar (Difco). Plates were incubated for 48 h at 35 C before counting.

Titratable acidity

Titratable acidity was expressed as ml of 0.1 N NaOH required to titrate to pH 7.0 a 10-ml aliquot of the liquid medium after centrifugation and dilution with 50 ml of distilled water.

RESULTS AND DISCUSSION

The liquid medium was devised to provide a broth in which production of lactic acid by the starter culture organisms could be measured without competition from the naturally occurring microflora found in meat. Lebanon bologna formulae differ so widely that a satisfactory model cannot be devised, but the test medium was made to provide pH value and sugar concentrations in the ranges found in these products, and the range of Lebanon bologna spice mixture concentrations tested encompassed the amounts of spice mixture used in bologna manufacture.

Titratable acidity data (Table 1) show a definite increase in acid production in all samples containing spice compared to the control sample without spice. However, increases in titratable acidity in spice-

containing samples were not commensurate with increasing spice concentration. Maximum acid production was obtained in samples containing black pepper, allspice, and the Lebanon bologna spice mixture at the 12 g/l level. With nutmeg, maximum production of acid was obtained at the 8 g/l level. Possibly, an increased concentration of this spice beyond the 12 g/l concentration might lead to inhibitory effects on the starter culture organisms.

The extent to which acid production was enhanced in the liquid medium depended on the spice used. For example (Table 1), after 96 h of fermentation, starting with an initial titratable acidity of 0.66 ml, the control reached a value of 4.15 ml; black pepper, 6.53 ml; allspice, 6.84 ml; nutmeg, 7.66 ml; and the spice mixture, 9.65 ml when 8 g/l of spice was used. The bacterial counts for the control and the spice containing samples were in a very narrow range, $1.4\text{--}2.0 \times 10^8$ cells/g. The high titratable acidity found in the broths containing the spice mixture might be attributed to effects of spices present in lesser quantities in the spice mixture, or it might be due to synergistic or additive effects of spices on the starter culture organisms.

To examine the effect of spice on growth of and acid production by the individual organisms, flasks with the liquid medium containing 8 g/l of Lebanon bologna spice mixture were inoculated with *L. plantarum* or *P. cerevisiae*, incubated and sampled for analyses as for the mixed culture. Initial counts were 2.6×10^4 cells/ml for *L. plantarum* and 1.8×10^4 cells/ml for *P. cerevisiae*. The results of the analyses are presented in Fig. 1. As with the mixed culture, bacterial count data showed that the spice mixture did not stimulate bacterial growth significantly. The spice mixture evoked a definite stimulation of acid production by both organisms, but *P. cerevisiae* did not produce an increase in titratable acidity as strongly as *L. plantarum*. After 96 h of incubation in the presence of 8 g/l of the spice mixture the titratable acidity values were 4.24 ml for *P. cerevisiae* and 10.27 ml for *L. plantarum* cultures, while the control values were 1.70

TABLE 1. Effect of black pepper, allspice, nutmeg, and lebanon bologna spice mixture on growth of and acid production by a mixed starter culture (*L. plantarum* and *P. cerevisiae*).

Spice,	g/l	Incubation time at 35 C							
		24 h		48 h		72 h		96 h	
		TA ^a	count ^b /ml	TA	count/ml	TA	count/ml	TA	count/ml
Control		2.08	4.1×10^8	3.00	3.5×10^8	3.70	4.9×10^8	4.15	1.5×10^8
Black pepper	4	2.91	6.6×10^8	4.74	8.8×10^8	5.73	3.3×10^8	6.48	2.2×10^8
	8	3.17	7.5×10^8	5.00	4.3×10^8	6.04	1.9×10^8	6.53	1.8×10^8
	12	3.10	6.5×10^8	4.92	6.4×10^8	6.30	3.1×10^8	7.00	1.7×10^8
Allspice	4	2.20	2.1×10^8	3.59	2.5×10^8	4.95	1.6×10^8	6.07	1.4×10^8
	8	2.25	1.8×10^8	3.84	3.2×10^8	5.52	2.4×10^8	6.84	1.4×10^8
	12	2.15	1.8×10^8	4.10	2.5×10^8	5.97	1.7×10^8	7.30	1.6×10^8
Nutmeg	4	2.39	5.0×10^8	4.40	5.5×10^8	5.75	3.2×10^8	7.02	9.0×10^7
	8	2.45	5.0×10^8	4.87	6.5×10^8	6.38	2.3×10^8	7.66	1.5×10^8
	12	2.88	4.7×10^8	5.00	4.8×10^8	6.30	2.5×10^8	7.23	1.0×10^8
Mixture	4	3.60	5.5×10^8	6.09	9.5×10^8	7.59	5.0×10^8	8.82	2.0×10^8
	8	3.78	6.6×10^8	6.80	7.7×10^8	8.50	9.0×10^8	9.65	2.0×10^8
	12	3.67	6.6×10^8	7.01	1.5×10^9	8.29	8.4×10^8	10.05	2.4×10^8

^aTA = Titratable acidity. The initial titratable acidity for the control samples was 0.66 ml.

The values for the spice containing samples were within 0.05 ml of the control value.

^bThe initial bacterial count was 3.0×10^4 cells/ml.

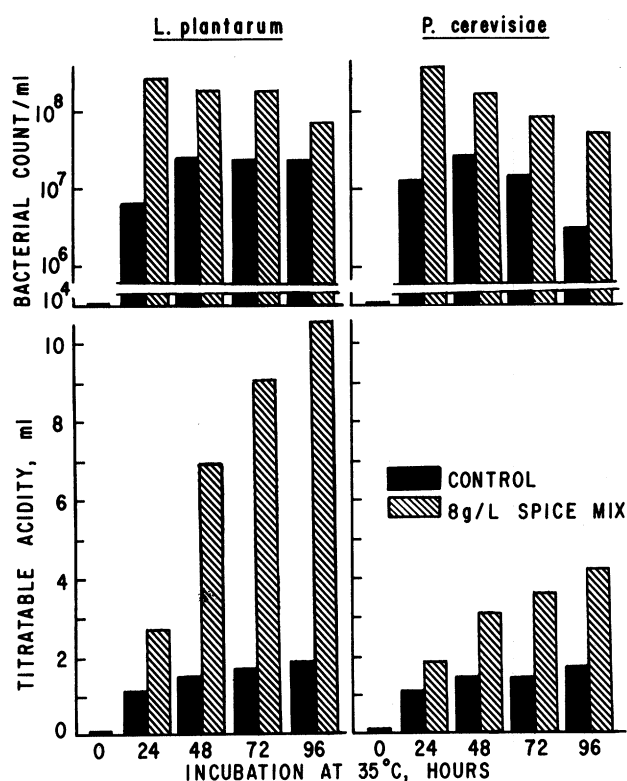


Figure 1. Effect of 8 g/l of Lebanon bologna spice mixture on growth of and acid production by *L. plantarum* and *P. cerevisiae*.

and 1.88 ml, respectively.

It can be concluded from this work that black pepper, allspice, nutmeg, and lebanon bologna spice mixture do not stimulate population growth of starter culture organisms, but do stimulate production of acid by these bacteria. Moreover, the multi-component spice mixture exerts a greater stimulatory effect on acid production by the starter bacteria than do the individual spices. Increasing concentrations of spice from 4 to 12 g/l brings about a slight increase in acid production except with

nutmeg which showed optimum stimulation of acid production at the 8 g/l concentration. Production of acid by both *L. plantarum* and *P. cerevisiae* was stimulated by all spices used, but acid production by *P. cerevisiae* is very low in comparison with that of *L. plantarum*.

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